Lasers in Surgery and Medicine 19:340–346 (1996)

Optimizing Light Dosimetry in Photodynamic Therapy of Early Stage Carcinomas of the Esophagus Using Fluorescence Spectroscopy

Daniel R. Braichotte, PhD, Jean-François Savary, MD, Philippe Monnier, MD, and Hubert E. van den Bergh, PhD

Institute of Environmental Engineering, Ecole Polytechnique Fédérale, CH-1015 Lausanne, Switzerland (D.R.B., H.E.v.d.B.); Head and Neck Surgery Division, Centre Hospitalier Universitaire Vaudois, CH-1011 Lausanne, Switzerland (J.-F.S., P.M.)

Background and Objective: **Under standardized conditions (drug and light dose, timing), the result of the photodynamic therapy (PDT) of carcinomas of the esophagus with tetra(meta-hydroxyphenyl)chlorin (mTHPC) shows large variations between patients.**
Study Design/Materials and Methods: **Before patients underwent PDT treatment, the mTHPC level was measured in the lesion, the normal surrounding tissue, and the oral cavity, with an apparatus based on fluorescence spectroscopy.**
Results: **The fluctuations in degree of tumor destruction between patients can be explained by individual variations in the mTHPC level in the mucosa of the esophagus. The patients showing the highest mTHPC fluorescence signal had also the highest response to PDT. Also, a correlation between the mTHPC level in the oral cavity and esophagus mucosa has been found.**
Conclusion: **PDT can be improved by measuring the mTHPC level in the esophagus or the oral cavity before treatment by fluorescence spectroscopy, and then by adjusting the light dose to be applied to the observed mTHPC level.**

Key words: mTHPC, light dosimetry, photobleaching, photosensitizer

INTRODUCTION

Photodynamic therapy [1,2] is a modality of cancer treatment based on the selective accumulation of a non-toxic photosensitizer (PS), in a tumor as compared to the healthy surrounding tissue. In this study the PS is mTHPC [3–6]. Upon irradiation with light at a wavelength that is absorbed by the PS, the latter becomes phototoxic and potentially destroys the tumor without major damage to the surrounding normal tissue. When applying a standard set of PDT parameters this ideal situation has been attained only for a fraction of the clinical treatments of early stage carcinomas of the bronchi and the esophagus with the drug mTHPC (unpublished results). However for the same set of experimental conditions (PS dose, fluence, fluence rate, wavelength, delay between injection, and PDT, etc.) some patients were undertreated (i.e., the lesion recurred locally) or overtreated (i.e., surrounding normal tissue was damaged to a significant extent). This variation in PDT efficacy from patient to patient probably has two major causes. First, the uptake of the PS is not always as selective as one might hope. This is the case in particular for in situ carcinoma of the bronchi and the esophagus. Second, the reaction of a tissue after irradiation is a function of several parameters, among which the light dose (which can be relatively well controlled), and the concentration of PS in the tissue to be treated,

Accepted for publication October 26, 1995.

Address reprint requests to D. Braichotte, LPAS-IGE, EPFL, CH-1015 Lausanne, Switzerland.

are the most important. The latter parameter depends on the amount of PS injected, which was the same for all patients. However, the local PS concentration at the time of PDT is in general unknown due to interpatient variation in pharmacokinetics. This implies that if one could measure the local PS concentration, or at least something related to it, just before the PDT treatment and change the light dose according to the observation one might be able to obtain a much more optimal treatment for each patient.

Hence an efficient, simple, non-invasive and non-destructive method for drug dosing is needed. Conventional extraction [7] and radiolabelling methods [8] must be ruled out being either time-consuming or difficult to use routinely in a clinical context. Furthermore it should be realized that whereas it would be valuable to have absolute mTHPC concentrations for this application, relative concentrations, when standardized between patients, are sufficient for the present purpose. Light induced fluorescence (LIF) [9] can meet these requirements and using standard endoscopic techniques can be applied to the mucosae of the hollow organs [10].

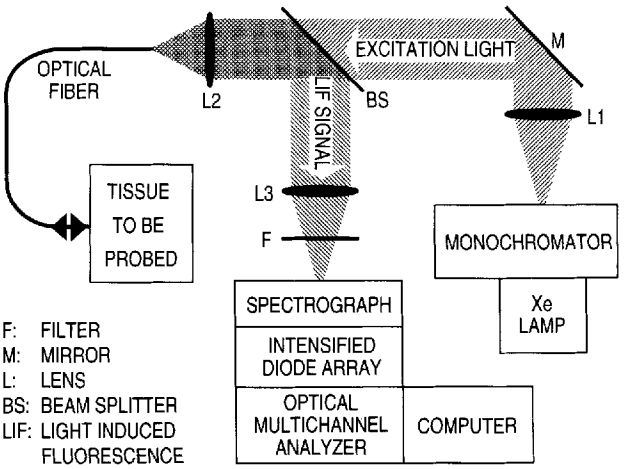

Fig. 1. Experimental set-up for clinical fluorescence measurements: Light from a Xe lamp is passed through a monochromator into an optical fiber by means of a microscope objective (L2). The fluorescence emitted by the tissue is collected by the same optical fiber, reflected by a dichroic mirror (BS), filtered (F) to remove the excitation light, and focused (L3) onto the entrance slit of a spectrograph coupled to an intensified diode array. The fluorescence spectra obtained is measured by an optical multichannel analyzer and processed by a personal computer.

MATERIALS AND METHODS

Patients

Sixteen patients participated in this study. All patients had an early stage (Tis or T1a) squamous cell carcinoma of the esophagus [11]. Four of them had a double tumor localization. All were intraveneously injected with 0.15 mg/kg of mTHPC. The LIF measurement used to establish the relative mTHPC concentration was made immediately prior to PDT. PDT was effected 4 days after injection of the drug.

Enrollment was voluntary and written consent was obtained from all patients, as defined in the protocol approved by the Ethical Committee of the CHUV hospital in Lausanne. A complete explanation regarding the potential side effects of mTHPC was given before enrollment and each patient received detailed written and oral instructions concerning photoprotective precautions.

Photosensitizer Preparation and Administration

mTHPC was kindly supplied by Scotia Pharmaceuticals Ltd. (Guildford, Surrey, England) as a powder and was stored in the dark at 4°C. Immediately prior to use mTHPC was dissolved in 20% ethanol, 30% polyethylene glycol 400, and 50% H_2O. The solution was then administered intraveneously through a bacterial filter under sterile conditions over a period of 10 minutes.

Instrumentation for LIF Measurements

The overall configuration of the fiber-based spectrofluorometric apparatus is shown in Figure 1. The desired excitation wavelength is selected by passing the light of a short arc xenon lamp through a monochromator. This excitation light is transmitted through a beam splitter prior to injection via a microscope objective in a 0.6 mm core diameter quartz optical fiber. The distal end of the fiber is positioned, for instance via the biopsy channel of an endoscope, on the surface of a superficial cancer or its neighboring normal tissue. The fluorescence spectrum induced in the tissue by light at the excitation wavelength is collected by the same optical fiber, reflected by the beam splitter, filtered to remove the excitation light, and focused onto the entrance slit of a spectrograph which is coupled to an intensified diode array. The signal obtained is displayed on the optical multichannel analyzer and can be further processed by a microcomputer.

The Experimental Protocol for LIF Measurements

mTHPC LIF signals measured just before PDT are obtained in the following way: The distal

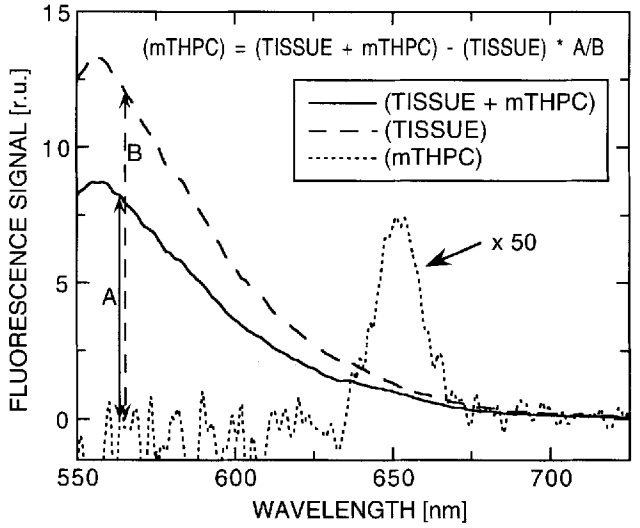

Fig. 2. Example of mTHPC LIF signal recovery. The tissue autofluorescecnce spectrum is subtracted from the mTHPC fluorescence spectra measured in the same tissue to give the fluorescence spectrum due to mTHPC alone. The latter is enlarged 50 times.

end of the fiber is positioned on the surface of a superficial cancer. The spectra obtained consist of both the autofluorescence spectrum of the tissue and the superposed fluorescence from the injected photosensitizer. Knowledge of the autofluorescence spectrum prior to injection allows for its subtraction to obtain the "pure" PS fluorescence spectrum before therapy. This enables the recovery of very small mTHPC LIF signals buried in the tissue autofluorescence background (Fig. 2). When the autofluorescence spectrum of the esophagus cannot be measured prior mTHPC injection, an average autofluorescence spectrum of the esophagus previously measured in different patients is used. In this case, a direct subtraction of the mean tissue autofluorescence spectrum is not possible, as its intensity varies from patient to patient. The mean tissue autofluorescence is at first scaled by a factor, which is the ratio between the LIF signal of the measurement composed of tissue autofluorescence and mTHPC fluorescence (A) and the mean tissue autofluorescence (B) at a given wavelength (Fig. 2). The latter is chosen so that there is no mTHPC fluorescence or blood absorption peak in this part of the spectrum.

The excitation and emission wavelengths are at respectively 420 nm and 650 nm. The power at the distal end of the probing fiber is of the order of 5 μW. The acquisition time per spectrum is about 1 second. No photobleaching can be detected at such low power levels and short acquisition times.

In order to compare the photosensitizer fluorescence between different measurements and between different patients, the power of the excitation light and the intensity of the fluorescence spectrum induced by this light in a fluorescent reference solution are measured. This allows us to correct for the variations of the excitation light intensity and slight changes in the optical alignment, for instance. The fluorescence intensities obtained in this way give a measure of the relative concentration of photosensitizer in tissue. It is assumed here that as the timing is always at 4 days after the injection the individual variations of the drug distribution in different tissue subcompartments can be neglected.

PDT Treatment and Response

PDT in the esophagus was carried out with an argon ion laser supplying the 514 nm light. The fluence rate was 100 mW/cm^2. Light was distributed homogeneously to the tumor surface by means of cylindrical light diffusers [12].

The degree of reaction of the treated area is determined 7 days after the treatment by endoscopic examination. The tissue reaction scale after irradiation has been defined in the following way: 0: no reaction; 1: petechial erythema; 2: necrotic area < irradiation window; 3: necrotic area = irradiation window; 4: necrotic area > irradiation window. One should note that this damage scale is neither completely quantitative nor linear. Reaction levels 0 and 1 lead to undertreatment of the lesion whereas reaction level 4 implies overtreatment at least on the surface of the esophagus wall and possibly also in some depth. This must be avoided, in particular in most hollow organs, in order to avoid complications like stenosis, stricture, or even fistulae.

RESULTS AND DISCUSSION

The mTHPC LIF signals from early squamous cell carcinomas in the esophagus of 16 patients have been measured just before PDT. All these patients were injected with 0.15 mg/kg and therapy was 4 days later. Figure 3 shows the results in form of a histogram. The vertical axis indicates the number of patients with an mTHPC fluorescence signal in a defined range. The size of the mTHPC fluorescence signal itself, in relative units, is given along the horizontal axis. The first thing that should be noted is that whereas the

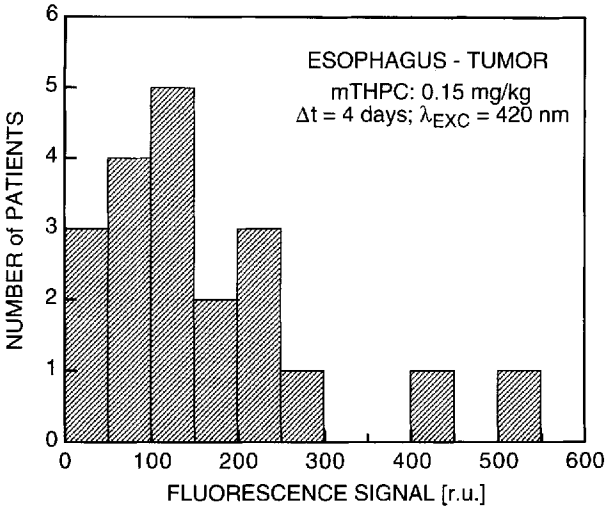

Fig. 3. Histogram of the number of patients which show an mTHPC LIF signal in a given intensity range measured prior to PDT on early squamous cell carcinomas in the esophagus. The patients were injected with 0.15 mg/kg of the drug. The delay between iv. injection and the LIF measurement which just precedes therapy is 4 days. Excitation is at 420 nm.

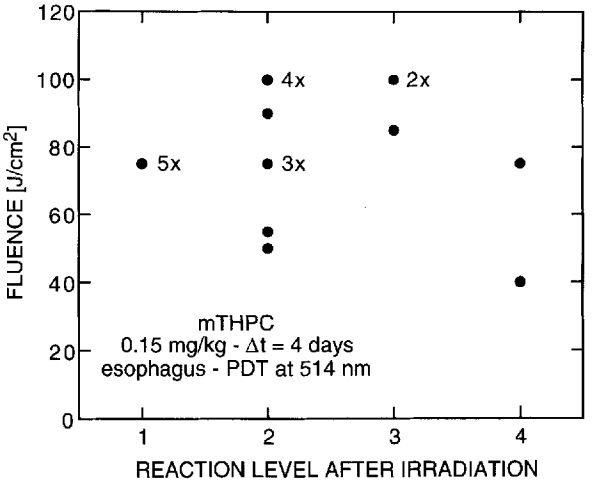

Fig. 4. The tissue reaction level 7 days after PDT as a function of the applied fluence during PDT. The PDT irradiation wavelength is 514 nm. Further conditions as in Figure 3.

mean of the mTHPC fluorescence signal is 160 relative units (median = 120), some patients exhibit about 3 times higher fluorescence, and some show a fluorescence signal up to 5 times lower. Thus, one observes very large fluctuations in the mTHPC concentration in the carcinomas at the time of PDT. This might be expected to some extent as not only the staging of these cancers is somewhat different leading to different uptake and removal of mTHPC. However, even for approximately equal tumor staging one would not necessarily expect equivalent pharmacokinetics in different patients. Similar results have been obtained for the bronchi and the oral cavity where differences of almost an order of magnitude between the lowest and the highest fluorescence signal have also been observed. The same effect, although to a lesser extent, has been seen for the PS used in most clinical studies, Photofrin [9,13–15].

Figure 4 represents the tissue reaction after therapy using the tissue damage scale indicated above as a function of the light dose applied in the PDT of these early stage squamous cell carcinomas of the esophagus wall. With such a plot we would like to be able to predict a specific tissue reaction level for a given light dose (i.e., one hopes for a reasonable correlation between applied fluence and reaction level). Unfortunately, this is clearly not the case. Typically, for one particular fluence, namely 75 J/cm^2, tissue reaction levels from 1 to 4 can be obtained. This means that some patients will be undertreated and some will be overtreated.

Figure 5 shows the tissue reaction level after therapy as a function of the applied light dose on the left vertical axis (□). This set of data is the same as that used in Figure 4. On the right vertical axis, the mTHPC fluorescence signal measured immediately prior to PDT, multiplied by the applied light dose during PDT is reported (●) as a function of the tissue reaction level after therapy. Note that by taking into account the mTHPC fluorescence signal, which is proportional to the mTHPC concentration [16], one can now see a reasonable correlation between the product of applied fluence and fluorescence signal on the one hand, and tissue reaction on the other hand. This means that type 1 tissue reactions (undertreatment) or type 4 (overtreatment) can now be avoided to a large extent by respectively increasing or decreasing the light dose applied to the lesion as a function of the observed mTHPC fluorescence signal. This procedure implies a significant improvement of the predictability of PDT outcome for early squamous cell carcinoma in the esophagus, where recurrence and side effects like stenosis or fistulae were not negligible previously [17].

Similar results have been obtained with PDT at 652 nm on early stage squamous cell carcinomas in the bronchi. Here however, adjusting the light dose to the mTHPC LIF signal in PDT may not be as important as in the esophagus due

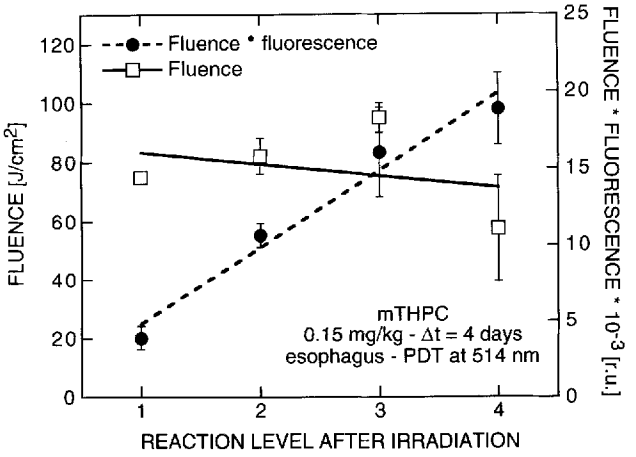

Fig. 5. The tissue reaction level 7 days after PDT as a function of the applied fluence (left vertical axis, □) and the applied light dose multiplied by the mTHPC fluorescence signal measured just before PDT (right vertical axis, •). The error bars represent 2 mean standard deviations. Further conditions as in Figure 4.

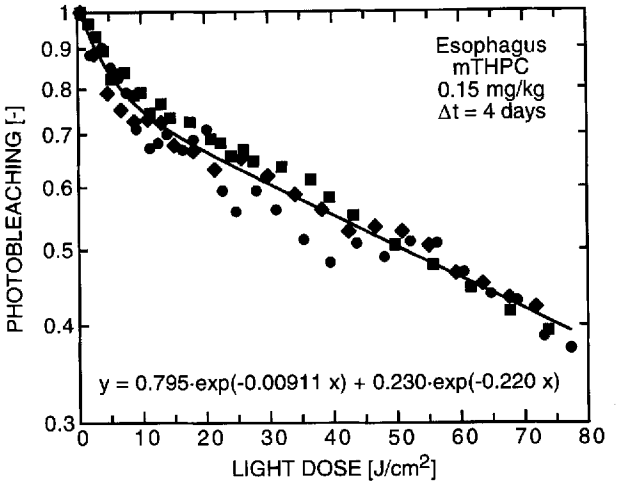

Fig. 6. The mTHPC photobleaching as a function of the fluence applied during the PDT treatment. Different symbols represent different patients. The mTHPC fluorescence decreases shows a clear double exponential shape. Further conditions as in Figure 4.

to the low uptake of mTHPC in the cartilage and in the connective tissue. This low uptake of mTHPC in the tissue underlying the bronchial mucosa acts as a zone which is protected against PDT damage [18]. Thus, in the case of the bronchi, slight overdosing of the applied fluence reduces the recurrence rate and does not imply a significant increase in side effects. PDT spot tests of 1 cm in diameter on the normal mucosa of the bronchi and the oral cavity have lead to similar results. One may conclude that in PDT under standardized conditions of injected drug dose, timing, and fluence rate, the fluence by itself does not allow for discrimination between the different levels of tissue reaction, whereas when combined with the fluorescence measurements, discrimination becomes possible in all these organs.

Some further improvements may still be possible: First, by using 520 nm excitation light instead of 420 nm for the mTHPC fluorescence measurements one should decrease their sensitivity to the tissue optical properties, in particular to the variation of blood content, thus improving their reliability.

Second, incorporation of the probing fiber in the light delivery system for PDT [12] will allow easier and more repeatable fluorescence measurements. This configuration also has another advantage: It permits to monitor in real time the photobleaching of the PS during PDT, which may also be an important parameter that may in the future be used to control more effective and safer photodynamic therapy. Such measurements are shown in Figure 6 for 3 patients. The photobleaching is expressed as the ratio between the mTHPC LIF signal induced by the 514 nm light used for PDT at time t = x and the mTHPC LIF signal at time t = 0 of PDT and is shown as a function of the applied fluence (fluence rate = 100 mW/cm^2). Under these conditions the overall behavior of the mTHPC photobleaching in the esophagus can be described by a biexponential equation: Photobleaching = 0.795 exp (−0.00911 F) + 0.230 exp (−0.220 F) where F is the applied fluence in J/cm^2. Typically a 50% mTHPC photobleaching is obtained with about 50 J/cm^2 of 514 nm light. The photobleaching rates obtained from these 3 patients are quite similar. These preliminary results would imply that this parameter could be ignored in the process of improving PDT, although some additional measurements would be useful to confirm this.

Third, preliminary clinical measurements of the optical properties of the esophagus wall have shown quiet large interpatient variations [19]. This would lead to a different energy density distribution in the tissue, and thus to different PDT efficiency. Such variations can also be measured in situ just before PDT and be corrected for using mathematical simulations. However, these interpatient variations of the tissue optical properties may not be important, as they are already included, at least partially, in the mTHPC LIF signal measured for monitoring PDT. This is partic-

ularly true when the same wavelength is used to perform PDT and LIF measurements, as the light propagation and distribution in the tissue will be almost identical. This should lead to a similar response of the PDT and LIF signals to variations of the optical properties of the tissue. For instance, in an esophagus mucosa with a higher than average blood content, the PDT light will be more absorbed by the hemoglobin. Hence, less light can be absorbed by the PS to induce the photochemical reaction, which in turn will lead to a decrease of the tissue reaction. The same arguments are also true for the fluorescence excitation light, also leading to a decrease of the PS LIF signal. Thus, if the PDT is monitored by the PS LIF signal, a higher PDT light dose could be applied to compensate for the blood absorption.

The apparatus used to measure the mTHPC LIF signal is relatively sophisticated. Also, measurements of the PS LIF signal in locations such as the esophagus (or other hollow organs) are not convenient for routine PDT therapies. This could be a drawback in the case of wider acceptance of PDT as a conventional cancer therapy. The estimation of the mTHPC level in the location to be treated from the mTHPC LIF obtained in an easily accessible location such as the oral cavity would greatly simplify the fluorescence measurements and eliminate the need for a relatively complex spectrofluorometer. Figure 7 shows such a correlation between the mTHPC LIF signal in the oral cavity and in the normal mucosa of the esophagus. We observe that the variations between the mTHPC LIF signal from the oral cavity and esophagus mucosa are much smaller than the interpatient variations. Thus, the mTHPC LIF signal in the oral cavity could help to give a reasonably accurate estimation of the mTHPC level in the esophagus.

Figure 8 shows the mTHPC LIF signal in the normal mucosa of the esophagus (●), and the corresponding mTHPC LIF signal in the carcinoma (□), as a function of the mTHPC LIF signal in the oral cavity. The average ratio of the mTHPC LIF signal between the carcinomas of the esophagus and the oral cavity, which corresponds to the slope of the dashed straight line, is 1.17. Similarly between the normal mucosae of the esophagus and the oral cavity (full line) we found an average ratio of 1.21. By dividing these 2 ratios, one obtained the average ratio of the mTHPC LIF signal between the carcinoma and the normal mucosae of the esophagus. This new ratio, which value is 0.97, is the mTHPC fluorescence selectivity. This

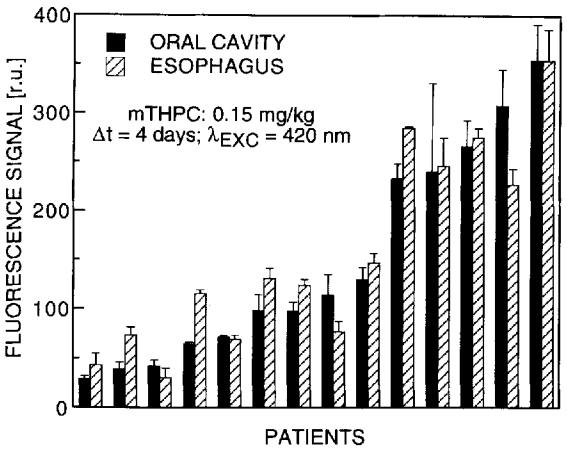

Fig. 7. A correlation of the mTHPC LIF signal measured in the esophagus and the oral cavity. The error bars represent 2 mean standard deviations.

shows that there is probably no selective mTHPC uptake 4 days after injection for early stage carcinomas of the esophagus. In a similar study with Photofrin an even lower value of 0.82 was found [9].

Expressing the tissue reaction level as a function of the applied light dose times the mTHPC fluorescence level has another advantage. It allows in principle to determine the influence of parameters such as the fluence rate, the relocalization of the PS in different compartments of the tissue or the cell, or to compare the sensitivity to PDT of different types of healthy mucosa and tumors, as the influence of the two major parameters (PS concentration and light dose) is cancelled.

CONCLUSIONS

Photodynamic therapy of early cancer with the second generation photosensitizer mTHPC, at the conditions currently applied, shows insufficient selectivity in uptake between tumor and surrounding normal tissue as compared to the interpatient variations in drug concentration. This implies that under so-called "standardized" conditions for PDT, some lesions may be undertreated leading to local recurrence, whereas other lesions and surrounding tissue may be overtreated leading to complications. It is demonstrated in this paper that by measuring the relative mTHPC concentration in the tissue by LIF at the time of PDT, and adjusting the light dose given in the therapy to the mTHPC fluorescence signal, one should be able to obtain more effective PDT with less side effects.

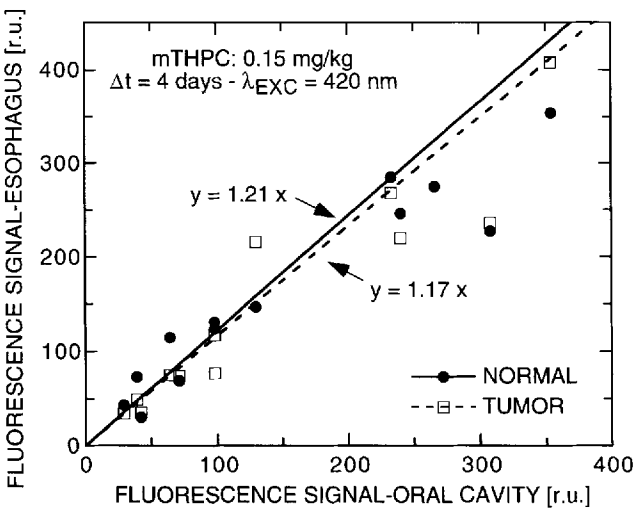

Fig. 8. The mTHPC LIF signal in the normal mucosae (•) and in the carcinomas (□) of the esophagus as a function of the mTHPC LIF signal in the oral cavity. Further conditions as in Figure 3.

ACKNOWLEDGMENTS

The authors are grateful to the Swiss National Science Foundation, the "Centre Hospitalier Universitaire Vaudois-University of Lausanne-Ecole Polytechnique Fédérale de Lausanne" Fund, the Swiss National Priority Program in Optics, and Scotia Pharmaceuticals for financial support.

REFERENCES

1. Henderson BW, Dougherty TJ. How does photodynamic therapy work. Photochem Photobiol 1992; 55:145–157.
2. van den Bergh H. Photodynamic therapy and photodetection of early cancer in the upper aerodigestive tract, the tracheobronchial tree, the esophagus and the urinary bladder. In: Amaldi U, Larsson B, eds. "Hadrontherapy in Oncology." Amsterdam: Elsevier Science B.V. 1994, pp 577–621.
3. Bonnett R, White RD, Winfield U-J, Berenbaum MC. Hydroporphyrins of the meso-tetra(hydroxyphenyl)porphyrin series as tumor photosensitizers. Biochem J 1989; 261:277–280.
4. Ris H-B, Altermatt HJ, Inderbitzi R, Hess R, Nachbur B, Steward JCM, Wang Q, Lim CK, Bonnett R, M.C. B, Althaus U. Photodynamic therapy with chlorins for diffuse malignant mesothelioma: initial clinical results. Br J Cancer 1991; 64:1116–1120.
5. Berenbaum MC, Bonnett R, Chevretton EB, Akande-Adebakin SL, Ruston M. Selectivity of meso-tetra(hydroxyphenyl)porphyrins and chlorins and of Photofrin II in causing photodamage in tumor, skin, muscle and bladder. The concept of cost-benefit in analysing the results. Lasers Med Sci 1993; 8:235–243.
6. Ma L, Moan J, Berg K. Evaluation of a new photosensitizer, meso-tetra-hydroxyphenyl-chlorin, for use in photodynamic therapy: A comparaison of its photobiological properties with those of two other photosensitizers. Int J Cancer 1994; 57:883–888.
7. Ris H-B, Altermatt HJ, Nachbur B, Stewart CM, Wang Q, Lim CK, Bonnett R, Althaus U. Effect of drug-light interval on photodynamic therapy with meso-tetrahydroxyphenylchlorine in malignant mesothelioma. Int J Cancer 1993; 53:141–146.
8. Folli S, Westermann P, Braichotte D, Pèlegrin A, Wagnières G, van den Bergh H, Mach J-P. Antibody-indocyanin conjugates for immunophotodetection of human squamous cell carcinoma in nude mice. Cancer Res 1994; 54:2643–2649.
9. Braichotte D, Wagnières G, Bays R, Monnier P, van den Bergh H. Clinical pharmacokinetic studies of Photofrin by fluorescence spectroscopy in the oral cavity, the oesophagus and the bronchi. Cancer 1995; 75:2768–2778.
10. Braichotte D, Savary J-F, Monnier P, van den Bergh H. Enhanced photodynamic therapy and dosimetry by light induced fluorescence. Proc Soc Photo-Opt Instr Eng 1995; 2371:120–124.
11. Beahrs OH, Henson DE, Hutter RVP, Kennedy BJ. "Manual for staging of cancer. (4th ed.)" Philadelphia: J.B. Lippincott, 1992.
12. Mizeret J, Thielen P, Theumann J-F, Bays R, Wagnières G, Savary J-F, Monnier P, van den Bergh H. New distributors for homogeneous and monitorable light delivery in photodynamic therapy. Proc Soc Photo-Opt Instr Eng 1995; 2323:58–69.
13. Brown SB, Vernon DI, Holroyd JA, Marcus S, Trust R, Hawkins W, Shah A, Tonelli A. Pharmacokinetics of Photofrin in man. In: Spinelli P, Dal Fante M, Marchesini R, eds. "Photodynamic Therapy and Biomedical Lasers." Amsterdam: Elsevier Science, 1992, pp 475–479.
14. Pantelides ML, Moore JV, Forbes E, Truscott TG, Blacklock NJ. The uptake of porphyrin and zinc-metalloporphyrin by the primate prostate. Photochem Photobiol 1993; 57:838–841.
15. Wang K, Gutta K, Densmore J. The use of laser induced fluorescence in the determination of photosensitizer concentration during photodynamic therapy of Barrett's esophagus. Lasers Surg Med 1995; S7:42.
16. Braichotte D, Savary J-F, Glanzmann T, Westermann P, Folli S, Wagnières G, Monnier P, van den Bergh H. Clinical pharmacokinetic studies of tetra(meta-hydroxyphenyl)chlorin in squamous cell carcinoma by fluorescence spectroscopy at two wavelengths. Int J Cancer 1995; 63: 198–204.
17. Savary J-F, Monnier P, Wagnières G, Braichotte D, Fontolliet C, van den Bergh H. Preliminary clinical studies of photodynamic therapy with meso-tetraydroxyphenyl chlorin (m-THPC) as a photosensitizing agent for the treatment of early pharyngeal, oesophageal and bronchial carcinomas. Proc Soc Photo-Opt Instr Eng 1993; 2078:330–340.
18. Lam S. Photodynamic therapy of lung cancer. Thorax 1993; 48:469.
19. Bays R, Wagnières G, Robert D, Mizeret J, Braichotte D, van den Bergh H. Clinical measurements of tissue optical properties in the esophagus. Proc Soc Photo-Opt Instr Eng 1995; 2324:39–45.